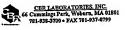
FAX COVER SHEET

Please deliver the following page(s) to:

TO: Mr Jack Benasera the spelling
TO: WIT Lack Tonapeta me spening
FAX NUMBER: 508-732-0400
FROM: Sherwin V Keuy, UD
FAX NUMBER: (781) 937-0799
TOTAL NUMBER OF PAGES: 7
MESSAGE: I apologize if some of the
enclosed material is a "rchash" of
what you already know- It is
done for completeness and gives
you the backround for the development
of the new product.
May Tacobon and I will be
meeting with Kevin at goo Huntington on
If there is a problem receiving this FAX, please contact: Munday - I will At (781) 938-3700. Extension: 1ef Jour know how to reach us
Sherwin



To Jack B From Dr SVK ce MSJ PhD

Prior to preventing some of the information you might need for "European Submission" - some backround might be helpful.

The new material is a coagulant

un that it minics the action of

thrombin. It can convert a pure

successive THEODEN

Saline suppension of Fibrinogen We

are in the process of further characterizing
the material (site of action shown

in the enclosed (figure 1- fibringen-7fibrin).

The material presently used to a

procoagulant. We have previously
demorphated at CBRL that the

active component is Xa (Factor Xa).

It initiates the coagulation pathways

as shown in figure 1. This material

*DIRECTLY Can not x convert fibrinogen to fabrin.

3. The mannifol that is combined with the ACD anticoagulant is well known to European regulatory agencies. It is a component of two ned cell preservative solutions SAGM and ADSOL. The mannitol is used in those preservative solutions to decrease micro ressicle shedding. It serves a similar purpose in our procedure and facilitates the separation of the precipitated proteins and red blood cells from the supernetant following centrifugation. The supernatant dantains the coaquilant. We have used the SmartPReP to prepare the congulant. The tubes we used to prepare the coagulant were of the same material but shorter in length to accommodate the internal dimensions of the Smarttle? Most of our studies with the proposed test tubes were done using stand alone centrifuges wing the same g force and time that we will have in both Smartthet sydems.

Preparation of the Congulant

The congulant is prepared from a whole blood sample that utilizes a modification of the fractionation procedure based on * Cohn's Method 6. All other procedures utilize plasma as the starting material and in most instances use cryoprecipitation as the first step in plasma fractionation.

Utilizing a proprietary level of ethanol at a pit of 6.8-7.2 in the a specified incubation time, the mixture is centrifuged. During this process the red blood cells and proteins such as fibringen, proteins and S and antithrombin III are precipitated. The precipitated proteins are superated from those in solution by a conventional liquid-solid separation technique such as centrifugation. We have characterized to some extent the proteins semoved and the content of the supernatant which is our coaquiant. Additional

Studies are on the drawing board which well: wfurther characterise the coagulant and its relationship to the amben; (2) characterize what proteins are contained in the precipitate and their quantification; (3) Petermine clotting times with both platelet concentrates (various levels) and platelet-poor-plasma using the existing 3:1 nates and possibly others; (4) Evaluate the growth factor release over time when the coagulant is combined with a platelet conventiate; (5) prepare the congulant in the ban-a Smart PRePresing the proposed test tubes and disposables.

Existing Characterization of the Coequiant

1) Clotting Studies			
Roto	Clotting	Time -	Second S
Platelet Congentrate: Coaquilant	Initial	1 hour	3hrs 5hrs
3 : 1			9.4 10.5
	±1.5	±0.7	±1.2 ± 1.8

n = 18

© Comparison of Coagulant	to Normal Plasma
Antithrombin III Reduce	tion 84,5/- n:22
Fibrinogen Reduction	100 ½ - n=16
Protein C Reducti	m 41,5%-n=5
Factor XIII Reduct	
Plasminogen Reduct	ion 43.0%- N=5
Prothranben Reduct	on $5.0\% - 11 = 2$
	eparation Scheme
8ml Whole Bloom	1 - ACD- Mannitol
1.6mL Add *95% E	thanol and 0.1 mL 10% Cacl = 1.7mL
	te the mixture- 30 min.
Centrifu	e in the Smart PREP
Separ by	ate Supernatur from Receptate Serum Separator Debite
Doi to to	Supernatant
Recipitate_ RBC's	Supernatant Coagulant "Albumin;"
Flbringen. Other Proteins	"Hothrombin"
Other riberts	
	77 I BELLEVE
	HE MEANT "THROMBIN"